OTS: 60-11,663

JPRS: 2694

4 June 1960

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- USSR -

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Distributed by:

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WASHINGTON 25, D. C.
~~Price: $0.30~~

U. S. JOINT PUBLICATION RESEARCH SERVICE
205 EAST 42nd STREET, SUITE 300
NEW YORK 17, N. Y.

JPRS: 2694

CSO: 4005-N

ATTENUATED VARIANT "M" OF RICKETTSIA BURNETI AS A POSSIBLE LIVE VACCINE AGAINST Q-FEVER

[This is a translation of an article written by V. A. Genig in Vestnik Akademii Meditsinskikh Nauk SSSR (Herald of the Acad. Med. Sci. USSR), Vol. 15, No. 2, 1960, pages 46-57.]

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During the past few years a number of experimental studies appeared in Soviet and foreign literature reporting variability phenomena among many species of rickettsiae pathogenic to humans. The present state of the problem of variability of rickettsiae is described thoroughly by P. F. Zdrodovskiy (1959) in his review of Soviet and foreign research.

Among the experimental works related to the variability of rickettsiae, those studies deserve the most attention which show the possibility of a considerable and stable loss of virulence. This may result either from splitting-off of rickettsiae during cultivation on chick embryos (Clavero and Gallardo, 1948), or from prolonged, continuous passing of rickettsiae under special conditions, as for example through lice (A. V. Pshenichnov and O. N. Sheveleva, 1954).

The Madrid strain "E" of R. prowazeki is an example of an altered variant of rickettsiae which split off from the initial strain in the process of cultivation on chick embryos. The "E" strain discovered by Spanish researchers Clavero and Gallard when Provazek rickettsiae, isolated from the blood of a typhus fever patient, was cultivated on chick embryos, is characterized as an apathogenic strain with altered but sufficiently preserved immunogenic properties toward guniea pigs. The "E" strain is now being studied and tested with a view toward its possible utilization as a live vaccine against typhus fever (Gallardo, Fox, 1948, Fox, etc., 1954, 1955, Zdrodovskiy, Golinevich, Yablonskaya, 1958).

The changes of properties in the process of cultivating on chick embryos is observed also on another species of rickettsiae -- R. burneti. For example, upon passing of "Nine Mile," "Christie," and other R. burneti through chick embryos, Stoker (1953) and Stoker and Fiset (1956, 1957) detected changes in their antigenic activity conditionally designated as variation-phases. Antigens prepared from egg cultures of the first four to seven passages reacted poorly to specific sera in complement fixation, in spite of a high rickettsia concentration, but showed well defined agglutination and antiglobulin sensitization. In the process of adaptation to chick embryos the antigenic activity of rickettsiae

increased. A reversed variation phase took place readily when rickettsiae adapted to chick embryos were introduced into various laboratory animals[1]. The change in the complement fixation activity of R. burneti during their adaptation stage to chick embryos has an important practical significance in the isolation and study of these various rickettsiae strains. This fact, apparently, explains the serologic dissimilarity of R. burneti strains observed by many researchers.

A number of investigations carried out on the causative agents of Rocky Mountain spotted fever showed that the virulence of many strains of Ricketts rickettsiae is very variable and that they can manifest themselves in mutually reversible phases: virulent and avirulent. Definite conditions were also ascertained under which changes of virulence of rickettsiae take place, as well as a transition from one phase of virulence to another. Thus, for example reduction of the virulence of Ricketts rickettsiae present in the D. andersoni ticks was observed during the process of moulting of ticks, or keeping infected ticks in a refrigerator, and, inversely, the rickettsia virulence is restored upon incubation of infected ticks for three days at + 37° C (Price, 1953; Price and Gilford, 1954).

Of substantial importance in the study of the variability of rickettsiae are biochemical investigations which have helped clarify a number of factors affecting the biological activity of rickettsiae. The studies of Price and Gilford (1955) on the causative agent of Rocky Mountain spotted fever demonstrated that the processing of rickettsiae in vitro with diphosphopyridinenucleotide (DPN) and coenzyme A contributes to the transition of rickettsiae from a nonvirulent phase to a virulent one, while incubation of rickettsiae with paraaminobenzoic acid leads to the loss of virulence. Analogous results were obtained by Bovarnick and Allen (1953, 1957). These authors demonstrated that the loss of biological activity of the "E" strain of R. provazeki (toxicity to mice, infectiousness to chick embryos, and hemolytic activity) after repeated freezing and thawing-out is related to the decrease of DPN concentration in rickettsia cells. The initial rickettsial activity can be restored to a considerable degree, or even fully, by subsequent incubation of rickettsiae in the presence of DPN and coenzyme A.

The above experimental data represent a great biological interest and have important practical significance in the study of the epidemiology, pathogenesis, and diagnostics of rickettsioses. Modern data obtained in the study of rickettsial variability reveal also the perspective of research on controlled variability, especially with the object of obtaining strains suitable for use as live vaccines.

In studying the Grit strain of R. burneti grown on chick embryos, we discovered in 1957 a variant with markedly reduced pathogenic properties toward laboratory animals. A short description of this variant,

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1) Analogous phenomena of phase variability of Burnet rickettsias in newly isolated strains were observed by Brezina, 1958; Moreli, Gerbec, 1959.

conditionally designated as variant "M", was given in a review article of P. F. Zdrodovskiy (1959).

The present work, the suggestion of Prof. P. F. Zdrodovskiy, represents the results of experimental investigations devoted to the study of the changed variant "M" of the Grit strain of R. burneti, as compared to the original Grit strain and the native strains of Burnet rickettsiae isolated by various authors from a number of objects.

We studied the pathogenic and immunogenic properties of the changed variant "M" of Grit strain of R. burneti by various methods of infection of laboratory animals, and we determined the stability of the attenuated rickettsial properties by provoking infection with cortisone and effecting continuous passages on guinea pigs.

Material and Methods of Investigation

The Italian-Greek Grit strain and three native strains used in the experiments were isolated in the Crimea from patients' blood -- 388, 59 (Bektimirov, 1954) -- and from R. bursa ticks -- Rvz 396 (Tarasevich, 1954). For the infection of animals and chick embryos we employed dry egg cultures prepared by standard procedure from a suspension of the yolk sac of the infected chick embryos with an abundant accumulation of rickettsiae. The cultures were desiccated in a vacuum according to our method. As a filler we used skimmed milk or, in some experiments, a five percent saccharose-buffer solution.

To obtain a complete and reliable characteristic of the cultural and pathogenic properties of the investigated R. burneti strains, we carried out simultaneously comparative titration of the same culture on chick embryos and on various animal species (guinea pigs and mice) and determined minimal rickettsial infection doses.

The chick embryos, six to seven days old, were infected through the yolk sac with culture suspensions in tenfold dilutions from 10^{-4} to 10^{-11}. At infection doses of 10^{-9} to 10^{-11} we used one subpassage, since at these doses in the initial infection we could not detect rickettsiae microscopically in the yolk sacs. We used for subpassages the yolk sacs of infected chick embryos which had survived the 12th to 13th day following infection.

We used in the experiments guinea pigs, mice, and rabbits. The guinea pigs received 0.5 to 1 milligram of egg culture subcutaneously in the testicles, and 0.1 ml epidermally; mice, 0.5 ml intravenously; and rabbits, 0.2 ml intradermally in doses corresponding to a culture dilution of 1:20 to 10^{-10}. In passing the virus on guinea pigs we used 20 percent suspension of the testicular tissue, or one millimeter of the infiltrates of infected animals.

In evaluating the results of the guinea pig infection we took into account: the general and local reaction (infiltrates), the presence of the virus in the blood (by means of biological tests on guinea pigs and chick embryos), in infiltrates and organs (microscopically), and the presence of specific complement fixation antibodies and postinfection

immunity.

The presence of infection in mice was determined according to the enlargement of the spleen and the accumulation of rickettsiae in it, and serologically by means of the complement fixation reaction. The toxic properties of R. burneti were assayed by an intradermal test on rabbits.

Results of Investigation

I. Variants of the Grit strain of R. Burneti upon cultivation of chick embryos

The Italian-Greek Grit strain (testicles of an infected guinea pig) was obtained at the Institute of Epidemiology and Microbiology imeni Gamaleya from Prof. M. P. Chumakov in January 1951. Subsequent cultivation of R. burneti of the Grit strain was carried out on chick embryos, age six to seven days, according to Cox.

We studied egg cultures of R. burneti Grit strain prepared from the yolk sacs of chick embryos of various passages -- 11th, 42nd, 43rd, 44th, 45th, 46th, 50th, 82nd, 85th, 86th, and native strains -- 59, 388, and 396.

As seen from Table I, experiments on the determination of minimal infecting doses of egg cultures of Burnet rickettsiae on chick embryos and female guinea pigs elicited the presence of two variants of Burnet rickettsiae, Grit strain, equally highly sensitive to chick embryos and distinguished by their pathogenic properties in animal tests.

I-variant of R. burneti, Grit strain, conditionally designated as "B" variant, is highly pathogenic to guinea pigs (cultures of the 11th, 42nd, 43rd, and 45th passages of Grit strains). II- variant of R. burneti of the same strain conditionally designated as "M" variant.

In our experiments the "M" variant was elicited on the 44th passage in chick embryos (obtained from V. F. Ignatovich). Having split off from the virulent strain on the 44th passage (egg culture of 1954), the 44-M variant continued to be well cultivated in the yolk sacs of chick embryos not differing in morphology and accumulation of rickettsiae in chick embryos from the "B" variant. The minimum infecting doses of egg cultures of Burnet rickettsiae, "B" and "M" variants, prepared by standard procedure from various passages on chick embryos, corresponded to 10-10 culture dilutions. At the same time, in infecting guinea pigs with the "M" variant of Burnet rickettsiae, the disease of the animals was considerably less pronounced in its clinical infection indices.

Table 1

Comparative titration of chicken cultures of various R. burneti strains on guinea pigs on guinea pigs and chick embryos

Experimental model	Infection indices	Infection titers according to microscopic, clinical, and serologic indices						
		Native strains			"B" variant Grit strain			
		59	398	306	11th passage	43rd passage	44th passage	45th passage
Chick embryos	Presence of rickettsias . . .	$>10^{-9}$	10^{-9}	$>10^{-9}$	10^{-10}	10^{-10}	10^{-10}	10^{-10}
Guinea pigs	General reaction .	10^{-8} $(10)^{-8}$	10^{-8} $(10)^{-9}$	10^{-9}	10^{-9} $(10)^{-10}$	10^{-9} $(10)^{-10}$	10^{-8}	10^{-8}
	Local reaction . . .	10^{-9}	10^{-8}	10^{-9}	10^{-9} $(10)^{-11}$	10^{-9} $(10)^{-10}$	10^{-8} $(10)^{-9}$	10^{-8} $(10)^{-9}$
	CFR/Complement fixation reaction/ on 30th day following infection	10^{-9}	$>10^{-9}$	$>10^{-9}$	10^{-10}	10^{-10} $(10)^{-10}$	10^{-9} $(10)^{-9}$	10^{-9} $(10)^{-9}$

		"M" variant Grit strain			
		44th passage	45th passage	85th passage	46th passage
Chick embryos	Presence of rickettsias	10^{-10}	10^{-9}	10^{-10}	10^{-10}
Guinea pigs	General reaction..	10^{-8} $(10)^{-8}$	10^{-8}	10^{-7} $(10)^{-}$	10^{-7} $(10)^{-8}$
	Local reaction...	10^{-8} $(10)^{-9}$	10^{-8} $(10)^{-9}$	10^{-8} $(10)^{-7}$	10^{-8} $(10)^{-8}$
	CFR/Complement fixation reaction/ on 30th day following infection	10^{-7} $(10)^{-9}$	10^{-7} $(10)^{-9}$	10^{-7} $(10)^{-9}$	10^{-7} $(10)^{-9}$

II. Pathogenic properties of "B" and "M" variants of Grit strains of R. burneti

The pathogenic properties of "M" variants of R. burneti were investigated in comparison with the virulent "B" Grit strain in the experiments of infecting guinea pigs, white mice, and rabbits.

The 44th and 45th passages of "M" variant were studied in greater detail, as initial passages of the changed variant and as more remote passages. The cultures of the virulent "B" variant of the 11th, 43rd, and 45th passages were used on chick embryos. The titer of the "B" and "M" variants used on chick embryos corresponded to a 10^{-10} dilution.

Guinea pigs. The sensitivity of guinea pigs to Burnet rickettsiae ("B" and "M" variants) were studied in subcutaneous, testicular, and epidermal infections.

The general reaction in guinea pigs, when subcutaneously infected with a virulent strain, was regularly observed at doses corresponding to a 10^{-8} dilution, and irregularly at 10^{-9} to 10^{-10} dilutions. Depending on the strength of the infecting dose, the fever reaction would begin on the fourth to sixth day and last six to fourteen days at 10^{-2} to 10^{-4} doses, and a one- to three-days fever on the fifteenth to twenty-sixth day, at minimum infecting doses of 10^{-8} to 10^{-9}.

In contrast to the virulent "B" strain, guinea pigs infected with "M" variant of Burnet rickettsiae would have a one- to five-day fever on the fourth to seventh day which was regularly manifested only at large infecting 10^{-2} to 10^{-3} doses. When doses of a 10^{-4} to 10^{-6} order were administered, the fever was absent, or an irregular one- to two- day rise of temperature was noted on the sixth to sixteenth day following infection.

At the seat of introduction of Burnet rickettsia cultures, there appeared in guinea pigs a local reaction, the intensity and duration of which in "B"and "M" variants were dissimilar.

In variant "B" the local reaction was always manifested at 10^{-2} to 10^{-9} doses, and irregularly at 10^{-10} dilution. It expressed itself as a hemorrhagic inflammation of the subcutaneous cellular layer and adjacent muscles involving either the entire half of the guinea pig body on the side of infection (with a dose of 10^{-2} to 10^{-4}), or part of the abdomen (at a dose of 10^{-6} to 10^{-9}). Infiltrates appeared at the onset of fever and lasted from two weeks to two months.

A local reaction in guinea pigs infected with "M" variant was regularly observed at 10^{-2} to 10^{-5} doses (duration from three to 12 days). In contrast to "B" variant, a diffuse hemorrhagic infiltration with formation of infiltrates around the inguinal lymphatic node and extending to the abdominal muscles was observed in the instance of large doses only (10^{-2} to 10^{-3}). At 10^{-6} to 10^{-7} doses, the local process was limited to slight subcutaneous hemorrhages, without formation of infiltrates.

At an autopsy of guinea pigs during various periods following infection with "B" variant (on the 7th, 12th, 14th, or 17th day),

rickettsiae were found in the smears from the infiltrates and regional lymphatic nodes at doses of 10^{-2} to 10^{-8} order up to the 17th day following infection (the length of observation). In an "M" variant, isolated rickettsiae in the infiltrate were detected only at large doses (10^{-1} to 10^{-2}) up to the 12th day following infection.

Parallel with the evaluation of clinical infection in indicators which, as seen from above, are slight in the "M" strain, the presence of the endured infection in guinea pigs was elicited serologically by means of the complement fixation reaction with an antigen from Burnet rickettsia.

When guinea pigs were infected with Burnet rickettsiae or the "B" variant, the serological infection indicators were manifested regularly at infection doses of 10^{8} to 10^{9}, and irregularyl at 10^{10} doses. The mean titer of antibodies fluctuated on the 30th day within 1:4216 to 1:8320 limits at 10^{-2} to 10^{-4} doses and 1: 145 to 1: 1174 at 10^{-8} to 10^{-9} doses.

The presence of the endured infection in guinea pigs infected with Burnet rickettsiae of the "M" variant was always manifested serologically at infection doses corresponding to a 10^{-7} culture dilution, and only in isolated cases to a 10^{-8} to 10^{-9} dilution.

The level of the titer of antibodies in guinea pigs infected with the "M" variant is considerably lower than in the infection with a virulent strain. Depending on the infection doses of rickettsiae, it fluctuated on the 30th day from 1:20 to 1:640 (mean titers: 1:66 to 1:112) at 10^{-2} to 10^{-3} doses, and 1:5 to 1:80 (mean titers: 1:11 to 1:20) at 10^{-6} to 10^{-7} doses. Thus, in a subcutaneous infection of guinea pigs with a virulent culture of Burnet's rickettsiae (Grit strain) in 10^{-2} to 10^{-9} dilutions the disease was usually accompanied by general and local reactions and serological infection.

When Burnet rickettsiae of the "M" variant of the same strain was introduced into guinea pigs, the general reaction (one to five days) was regularly observed at doses corresponding to 10^{-2} to 10^{-3} culture dilution, and a local reaction at 10^{-5} dose. When the "M" variant was introduced in 10^{-6} to 10^{-7} doses, the infection in guinea pigs proceeded, in most instances, without symptoms and manifested itself maninly serologically.

Upon testicular and cutaneous infection with the "M" variant of Burnet rickettsiae, the clinical and serological infection indicators in guinea pigs were also less marked than in the "B" variant.

For instance, in cutaneous infection with a virulent "B" variant the disease in guinea pigs proceeded with a marked fever (lasting four to nine days) at 10^{-2} to 10^{-3} doses, and with a one- to four-day fever on the 7th to 23rd day at 10^{-5} to 10^{-6} doses.

In a similar infection method with the "M" variant, the disease in guinea pigs proceeded in most cases in an asymptomatic form and manifested itself serologically in the complement fixation test with an antigen from Burnet's rickettsiae at 10^{-2} to 10^{-4} doses. The level of titers

of antibodies in cutaneous infection with the "M" variant, as well as with other methods of infection is considerably lower than in infection with a virulent variant; at 10^{-2} to 10^{-4} doses it fluctuated between 1:5 to 1:80.

White mice. The sensitivity of white mice to Burnet rickettsia of the "B" and "M" variants was studied by intravenous infection with egg cultures in 1:20 to 10^{-9} dilutions. The tests were made parallel with two native strains -- 388 and 59.

The infection in mice upon contamination with all the above-mentioned strains proceeded asymptomatically. A characteristic indicator of the infection in mice was the enlargement of the spleen, accumulation of rickettsiae in it, and the presence of specific antibodies with a maximum titer level on the 18th to 24th day following infection. However, the expression of the above symptoms of infection differed with various strains depending on their virulence. In variant "B" the disease in mice with clearly pronounced enlargement of the spleen and rickettsiae in it was always elicited at a dose corresponding 10^{-4} dilution, and only in isolated cases at 10^{-8} to 10^{-9} doses.

Serologically the presence of infection was regularly determined at 10^{-6} to 10^{-7} doses, and irregularly at 10^{-8} to 10^{-9} doses. Maximum level of antibodies was noted on the 18th to 24th day depending on the strength of the infection doses and fluctuated within the 1:320 to 1: 1280 limits at 10^{-2} dose, and within 1:40 to 1:60 at the 10^{-6} dose.

Data obtained upon infection of mice with native 59 and 388 strains are basically analogous to the results on mice infected with the "B variant, the only difference being that the antibody titers of mice infected with native strains were somewhat lower than in the infection with the "B" variant of grit strain.

Different results were obtained upon the study of "M" variant. The disease in mice was accompanied by a marked enlargement of the spleen and the accumulation of rickettsiae in it, observed only at doses corresponding to a culture dilution of 1:20 to 10^{-2}. As to the serological indicators, the infection in mice was elicited at 10^{-4} dose, and in isolated cases at a 10^{-6} dose. The level of antibody titers, when compared to "B" variant, was considerably lower and fluctuated within 1:10 to 1: 160 limits at 10^{-2} to 10^{-3} doses and 1:10 to 1:20 at a 10^{-4} dose. Thus, in tests on white mice the "M" variant of Burnet rickettsiae proved to be less pathogenic as compared to "B" variant.

Rabbits. The intradermal reaction in rabbits was achieved when Burnet rickettsiae were introduced into a 10^{-2} to 10^{-6} dilution. However, the local reaction with the "M" variant was somewhat weaker than the one with "B" variant. Thus, upon introduction of "B" variant a clearly pronounced intradermal reaction developed on the first to second day at 10^{-2} to 10^{-4} doses (the presence of infiltrates in the form of prominent solid and hyperemic nodes). On the 7th to 8th day a tissue necrosis was observed at a 10^{-2} dose.

In the "M" variant clearly pronounced infiltrates appeared in the diameter, upon intradermal introduction of rickettsiae in 10^{-2} to 10^{-3}

dilutions. The tissue necrosis was not observed at these doses. Upon introduction of cultures in 10^{-4} to 10^{-6} dilution, a slight erythema of the skin appeared at the site of injection.

Thus, the obtained data of a comparative study of "B" and "M" variants of R. burneti on laboratory animals attest to the fact that the "M" variant possesses considerably lower pathogenic properties.

In subsequent experiments we checked on the stability of the diminished pathogenic properties of R. burneti of the "M" variant during continuous passage of the virus on guinea pigs highly sensitive to Q-rickettsiosis, and also determined the resistance of the asymptomatic forms of infection in guinea pigs caused by the "M" variant after infection was provoked by cortisone.

Passage of R. burneti on Guinea Pigs

Continuous passages of R. burneti on guinea pigs were effected by introducing into the skin or testicles emulsions from infiltrates or testicular tissue obtained from guinea pigs at the height of the disease.

On the passage of the "B" variant virus on guinea pigs, the infection, with fever of 8 to 13 days durations, a diffuse hemorrhagic infiltration, and copious accumulation of rickettsiae in the infiltrate in subcutaneous contamination took place easily and apparently in an unlimited number of passages.

In the passages of "M" variant of R. burneti on guinea pigs, the disease with fever lasting one to three days in subcutaneous contamination took place in the first three passages only. In the subsequent three to four passages, the disease proceeded asymptomatically, there were no ricketssiae found in the smears from testicles or infiltrates, and on the seventh passage the disease was not elicited even serologically.

The Effect of Cortisone on the Course of Infection Caused in Guinea Pigs by "B" and "M" Variants of R. burneti

The effect of cortisone on the course of infection in guinea pigs was studied with the "B" and "M" variants of R. burneti. The infection of animals was carried out with dry standard egg cultures, the minimal infecting doses of which in subcutaneous contamination corresponded to the culture dilution of 10^{-9} in the "B" variant and 10^{-7} in the "M" variant. The titer of cultures on chick embryos corresponded to a 10^{-10} dilution. The egg cultures of R. burneti were introduced into the guinea pigs subcutaneously (one ml) in 10^{-2} to 10^{-3} ("M" variant) and 10^{-4} to 10^{-8} ("B" variant) dilutions.

Cortisone (French manufacture) was introduced in the animals intramuscularly using five mg twice a day for three days prior to infection, and 8 to 12 days after infection depending on the dose of rickettsiae. A total of 110 to 150 mg of cortisone was given each

guinea pig.

The evaluation of the results of the infection was done according to the following indices: the presence of a general and local reaction, the intensity and duration of rickettsiemia (by means of parallel biological blood tests on guinea pigs and chick embryos), the presence of rickettsiae in smears from various organs, and the emergence of specific antibodies in the blood.

"B" variant. Upon infecting with the "B" variant of R. burneti in 10^{-4} to 10^{-8} doses, the course of experimental Q-rickettsiosis in guinea pigs treated with cortisone was aggravated at large (10^{-4}) as well as small (10^{-8}) infecting doses. The clinical picture of the disease in guinea pigs given cortisone differed from that of the controls and was characterized usually by a lengthening of the fever period, absence of a local reaction, a rickettsiemia more marked in intensity and duration the presence of a large number of rickettsiae at the site of introduction (the subcutaneous cellular tissue), in the regional nodes, and in the spleen, liver, and kidneys.

The level of antibody titers of the blood during the first days of their appearance (13th to 14th day at 10^{-4} dose) in guinea pigs treated with cortisone was equal to or lower than the corresponding one in controls, though the infection in guinea pigs of the "cortisone group," as indicated above, proceeded with more intensity. During the latter stages of the disease (25th to 30th day) higher levels of antibodies were observed in animals treated with cortisone.

"M" variant. Upon subcutaneous introduction of the "M" variant of R. burneti in guinea pigs at a 10^{-2} dose, the general and local reaction in some of the animals treated with cortisone was completely inhibited or emerged during later stages than in the controls. The virus in the blood of animals treated with cortisone and in the controls was detected on the seventh day following infection. The determination of rickettsiae in the blood within 12, 17, 25, and 30 days following infection with parallel tests on guinea pigs and chick embryos brought negative results. The microscopic examination of infiltrates, regional lymph-nodes, and various organs of the animals of the "cortisone group" revealed rickettsiae in a limited number up to the 17th day following infection, and in the control animals up to the 12th day. The level of antibody titers in the blood of test guinea pigs as with the "B" variant exceeded considerably the level of antibodies of the controls.

Upon introduction of the "M" variant of R. burneti at a 10^{-5} dose the disease proceeded asymptomatically in the experimental and control groups of guinea pigs. The local reaction was absent in guinea pigs treated with cortisone. No rickettsiae were detected in the blood nor in smears from regional lymph nodes and various organs of the control or experimental groups. The titers of blood antibodies of guinea pigs treated with cortisone were higher on the 25th to 30th day than in the controls.

Thus, guinea pigs infected with the culture of "M" variant showed as a result of cortisone treatment, only a mild increase of the infection at high infecting doses (10^{-2}), which indicates the markedly reduced

pathogenic properties of this variant.

Thus, the attempts to provoke infection by means of cortisone showed that the "M" variant retains its ability to cause asymptomatic forms of infection in guinea pigs whose natural defense under the influence of cortisone was markedly reduced. Nor was there enhancement of infection in animals under conditions of passing the "M" variant rickettsiae through guinea pigs.

III. Antigenic Properties of "B" and "M" Variants of R. burneti

We studied the antigenic properties of "B" and "M" variants in the complement fixation reaction by cross-titration of antibodies with homologous and heterologous sera.

For serological examinations we employed the corpuscular antigens of the "B" and "M" variants in 250 million per milligram concentration, as per the bacterial optic standard, and immune Burnet ricksttsiae sera obtained from guinea pigs infected with egg cultures of "B" and "M" variants in a 1:100 dilution.

The results showed that Burnet rickettsiae of the "B" and "M" variants do not differ from each other in the complement fixation reaction and can be utilized equally in this reaction. Parallel with these observations, the studies of immune sera of Burnet rickettsiae of the "B" and "M" variants show a marked difference in the level of antibody titers between themselves, apparently conditioned by the difference in the pathogenic and immunogenic properties of these variants.

IV. Immunogenic Properties of the "M" Variant of the Grit Strain of Burnet rickettsiae

In studying the immunogenic properties of the "M" variant of Burnet rickettsiae it was important to determine the minimum immunizing doses of rickettsiae, the speed of appearance of immunity, and its intensity and duration. To determine the minimum immunizing doses of Burnet rickettsiae, guinea pigs were subcutaneously infected with various doses (10^{-2} to 10^{-10}) of "B" and "M" variants; they were tested for immunity within 45 days following infection by subcutaneous injection of 1,000 to 1,000,000ID Burnet rickettsiae of a virulent Grit strain.

As shown in Table 2 the minimum immunizing doses of Burnet ricksttsiae of "B" and "M" variants for guinea pigs (on testing within 45 days) correspond to the liminal infecting doses of "B" and "M" variants. Thus, complete immunity to 1,000 ID within 45 days following infection was manifested in guinea pigs who had been initially infected subcutaneously with 10^{-9} to 10^{-10} doses of "B" variant, and 10^{-8} doses of "M" variant.

To determine the intensity of the postinfection immunity in guinea pigs infected with various doses of "B" and "M" variants, 1,000,000 ID Burnet rickettsiae of a virulent Grit strain were injected subcutaneously. As seen from Table 2, within 45 days following infection complete immunity

Table 2

Postinfection immunity in guinea pigs 45 days after their infection with "B" and "M" variants of R. burneti

Variants of Grit strain	Doses in initial infection / Immunity / Doses in secondary infection	10^{-2}			10^{-3}—10^{-4}			10^{-5}—10^{-6}		
		complete	partial	absent	complete	partial	absent	complete	partial	absent
43rd passage «B»	1 000									
44th passage «B»	1 000	2	0	0	2	0	0	2	0	0
44th passage «M»	1 000	2	0	0				3	0	0
85th passage «M»	1 000	4	0	0	6	0	0	9	0	0
43rd passage «B»	1 000 000							2	0	0
44th passage «B»	1 000 000				2	0	0	3	0	0
44th passage «M»	1 000 000	3	0	0	0	2	0	3	2	0
85th passage «M»	1 000 000	3	5	0	2	2	0	2	3	0

Variants of Grit strain	Doses in secondary infection	10^{-7}—10^{-8}			10^{-9}—10^{-10}		
		complete	partial	absent	complete	partial	absent
43rd passage "B"	1 000	3	0	0	4	0	1
44th passage "B"	1 000	5	0	0	4	0	0
44th passage "M"	1 000	5	0	0			
85th passage "M"	1 000	9	0	0	2	1	0
43rd passage "B"	1 000 000	4	4	0	3	4	0
44th passage "B"	1 000 000	6	3	0	1	2	2
44th passage "M"	1 000 000	0	7	0	0	4	1
85th passage "M"	1 000 000	1	6	0	0	0	2

was manifested in all guinea pigs infected with the culture of variant "B" in 10^{-5} to 10^{-6} doses, a complete and partial immunity following infection with 10^{-7} to 10^{-8} doses, and irregular complete and partial immunity was noted at 10^{-9} to 10^{-10} doses. In guinea pigs infected with rickettsiae of the "M" variant at 10^{-2} to 10^{-6} dilutions, a complete and partial immunity was observed, and at 10^{-7} to 10^{-8} doses, mainly a partial immunity.

Thus, the data showed that the "M" variant possesses considerable immunogenic properties. As compared to the "B" variant, the immunogenicity of M" variant is somewhat weaker; these differences, however, were manifested only in testing the immunity with massive rickettsia doses.

To ascertain minimum periods for the appearance of the immunizing effect and its duration in guinea pigs infected with the "M" variant, a special experiment was conducted in which the immunity of animals was tested during the early stages -- on the 3rd, 7th, and 14th day and within 45 days, three months, and six months following infection.

An egg culture of the "M" variant vacuum-dried in a five percent saccharose buffer solution of 10^{-2} to 10^{-5} dilutions was used for the immunization of guinea pigs. The minimum infecting dose of the chick embryo culture corresponded to a 10^{-10} dilution (the titer of the culture was determined with one subpassage). The liminal infecting doses of the culture for guinea pigs infected subcutaneously corresponded to a 10^{-7} to 10^{-8} dilution, according to serological indicators.

The immunization of animals was carried out by means of subcutaneous injection of 0.5 ml of the culture in 10^{-4} to 10^{-6} dilution, or by placing 0.1 ml. of culture suspension in similar dilutions on the surface of the scarified skin with subsequent rubbing-in of the culture into the damaged skin. A total of 180 guinea pigs was immunized.

On subcutaneous immunization at a 10^{-4} dose, 14 guinea pigs out of 38 observed for 30 days showed a one- to three-day rise in temperature on the sixth to tenth day following injection. In all animals there appeared slight infiltrates at the injection site of a small edema of the subcutaneous cellular tissue.

With a 10^{-6} dose, the infection in guinea pigs proceeded asymptomatically. An autopsy of the guinea pigs showed small hemorrhages at the site of the culture-injection with no infiltration of the subcutaneous cellular tissue.

Upon epidermal immunization with a culture in 10^{-4} to 10^{-6} doses, the infection also proceeded asymptomatically in most cases. Some guinea pigs showed a one- to two-day rise of temperature at various periods following infection.

The determination of immunity in guinea pigs at early periods following immunization with live "M" variant culture showed that, within three days after a subcutaneous injection of the culture in 10^{-4} dose, all guinea pigs revealed a complete or partial immunity to 1000 ID of a virulent Burnet rickettsia culture. Within a week after the injection, all guinea pigs infected subcutaneously with a 10^{-4} dose showed complete immunity to 1000 ID, retained by the majority of animals for six

months (the period of observation).

Subcutaneous infection with a 10^{-6} dose culture brought similar results. Only a slight retardation of immunity formation was observed during the early stages (three to seven days).

Upon epidermal immunization, an earlier appearance of immunity (on the third day following infection) was noted in guinea pigs infected with a live 10^{-4} dose culture. The immunity obtained at this dose remains stable up to three months. At a 10^{-6} dose, the immunity was weaker and developed at later periods (on the 14th day).

Repeated infection of guinea pigs with massive doses of virulent Burnet rickettsiae within 45 days after immunization with a live "M" variant attest to the high immunogenicity of this variant. Immunity induced by the "M" variant was characterized in guinea pigs by early emergencey, considerable intensity, and definite duration (three to six months observation period).

Conclusions

1. Comparative study of various strains of R.burneti (Grit strains 59, 388, 396) in experiments on chick embryos and laboratory animals (guinea pigs, white mice, and rabbits) elicited a Grit strain variant which we designated as an "M" variant with reduced pathogenic properties, but sufficiently well preserved immunogenic properties.

2. The infectiousness of egg cultures of R. burneti of the "M" variant and the virulent "B" variant of the Grit strain to chick embryos was equivalent (EID = 10^{-10}).

3. Experiments on guinea pigs (subcutaneous infection) demonstrated a markedly lower intensity of clinical manifestations of infection with the "M" variant as compared to the "B" variant, with regularly elicited serological indicators of infection within 10^{-7} dose limits in "M" variant and 10^{-9} in "B" variant.

4. White mice are considerably less sensitive to R. burneti "M" variant than to "B" variant Grit strain.

5. When infection was provoked in guinea pigs by cortisone the stability of asymptomatic forms of infection caused by "M" variant was elicited. The "M" variant retained its acquired characteristics also after continuous passage on guinea pigs.

6. Serologically, the "M" variant is distinguished by its propensity to cause the formation of antibodies in laboratory animals (guinea pigs, mice) at a lower level as compared with the "B" variant of Grit strain.

7. The antigens from rickettsiae of "M" and "B" variants proved identical in the complement fixation reaction.

8. In addition to reduced pathogenic properties the "M" variant preserved demonstrable immunogenic properties. In experiments on guinea pigs the "M" variant showed good immunizing effect in the subcutaneous employment of 10^{-6} to 10^{-7} doses and in epidermal vaccination with a 10^{-4} dose.

9. The experimental studies conducted justify the study of the R. burneti "M" variant as a live vaccine against Q-fever in humans.

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